

AMENDED CLAIMS

AP20 Rec'd PCT/PTO 16 MAY 2006

[received by the International Bureau on 25 April 2005 (25.04.2005);
original claims 1-26 replaced by amended claims 1-26 (4 pages)]

1. A chimeric gene comprising the following operably linked DNA:
 - (a) a plant-expressible promoter;
 - (b) a DNA region which when transcribed yields a double-stranded RNA molecule capable of reducing the expression of an essential gene of a plant sap-sucking insect, said RNA molecule comprising a first and second RNA region wherein:
 - (i) said first RNA region comprises a nucleotide sequence of at least 19 consecutive nucleotides having at least about 94% sequence identity to the nucleotide sequence of said endogenous gene ;
 - (ii) said second RNA region comprises a nucleotide sequence complementary to said at least 19 consecutive nucleotides of said first RNA region;
 - (iii) said first and second RNA region are capable of base-pairing to form a double stranded RNA molecule between at least said 19 consecutive nucleotides of said first and second region;
 - (c) optionally, a 3' end region comprising transcription termination and polyadenylation signals functioning in cells of said plant.
2. The chimeric gene of claim 1, wherein said essential gene of said plant sap-sucking insect is selected from the group consisting of the genes encoding the following: a gut cell protein, a membrane protein, an ecdyson receptor, a vATPase, an amino acid transporter, a transcription factor, a peptidylglycine alpha-amidating monooxygenase, a cystein protease, an aminopeptidase, a dipeptidase, a sucrase/ transglucosidase, a translation elongation factor, the eucaryotic translation initiation factor 1A, a splicing factor, an apoptosis inhibitor, a tubulin protein, an actin protein, an alpha-actinin protein, a histone, a histone deacetylase, a cell cycle regulatory protein, a cellular respiratory protein; a receptor for an insect-specific hormonal signal, a juvenile hormone receptor, an insect peptidic hormone receptor; a protein regulating ion balance in the cell, a proton-pump, a Na/K pump, an intestinal protease; an enzyme involved in sucrose metabolism, a digestive enzyme, a trypsin-like protease and a cathepsin B-like protease.
3. The chimeric gene of claims 1 or 2, wherein said double-stranded RNA silences the gene corresponding to the DNA sequence of any one of SEQ ID NO: 5 to 8, SEQ ID NO: 11 or SEQ ID NO: 12.

4. The chimeric gene of any one of claims 1 to 3, wherein between said first and second RNA region, a spacer region containing a plant intron is present.
5. The chimeric gene of any one of claim 1 to 4, wherein said essential gene has a portion which occurs with the same sequence or with at least 94 % sequence identity in homologous genes of several plant sap-sucking insects.
6. The chimeric gene of any one of claim 1 to 5, wherein said promoter is a constitutive promoter.
7. The chimeric gene of any one of claim 1 to 6, wherein said promoter is a vascular-specific or a phloem-specific promoter.
8. The chimeric gene of claim 7, wherein vascular- or phloem-specific promoter is selected from the group consisting of: the rolC or rolA promoter of *Agrobacterium rhizogenes*, the promoter of the *Agrobacterium tumefaciens* T-DNA gene 5, the rice sucrose synthase RSs1 gene promoter, the *Commelina* yellow mottle badnavirus promoter, the coconut foliar decay virus promoter, the rice tungro bacilliform virus promoter, the promoter of the pea glutamine synthase GS3A gene, the *invCD111* and *invCD141* promoters of the potato invertase genes, the promoter isolated from *Arabidopsis* shown to have phloem-specific expression in tobacco by Kertbundit et al (1991), the VAHOX1 promoter region, the pea cell wall invertase gene promoter, an acid invertase gene promoter from carrot, the promoter of the sulfate transporter gene *Sultr1;3*, the promoter of a plant sucrose synthase gene, the promoter of a plant sucrose transporter gene.
9. A plant cell, tissue, or a plant or a plant seed comprising the chimeric gene of any one of claims 1 to 8 or the double-stranded RNA molecule described in any one of claims 1 to 8.
10. A method to silence a gene of a plant sap-sucking insect, comprising applying to the feed of said plant sap-sucking insect unpackaged naked dsRNA or siRNA which is targeted to an essential plant sap-sucking gene.
11. The method of claim 10, wherein said essential gene is any of the genes listed in claim 2 above.

12. The method of claim 10, wherein said application is by expression of a dsRNA chimeric gene in phloem cells of a plant.
13. A method to silence a gene in an plant sap-sucking insect, comprising: adding naked, unpackaged dsRNA or siRNA to the diet or feed of said plant sap-sucking insect, wherein said dsRNA or siRNA targets said gene.
14. A method of controlling plant sap-sucking insects, comprising expressing in the phloem of a plant dsRNA that targets an essential plant sap-sucking insect gene.
15. The method of claim 14 wherein said gene is a gene expressed at least in the intestine or in gut cells.
16. The method of claim 14 wherein said plant sap-sucking insect is an aphid or a whitefly.
17. A plant, comprising stably inserted in its genome, the chimeric gene of claim 1, so that said chimeric gene is expressed in the phloem or xylem of said plant.
18. A method of identifying gene function in a plant sap-sucking insect, comprising the step of applying naked, unpackaged dsRNA targeting a plant sap-sucking insect gene to the diet of said insect, and evaluating phenotypic or biochemical changes in said insect.
19. A method of identification of novel targets for insecticidal compounds, comprising the steps of: a) applying naked, unpackaged dsRNA or siRNA molecules to the feed or diet of a plant-sap sucking insect; b) analyzing which genes when silenced confer lethality to said insect, c) cloning and characterizing said genes thus analyzed; d) identifying compounds that disrupt or inactivate said gene or the RNA or protein encoded thereby; and e) contacting said compounds with said insect or feed or diet of said insect to confirm the pesticidal nature of said compound.
20. Phloem of a plant, comprising siRNA targeted to an aphid essential gene.
21. Phloem sap of a plant, comprising siRNA targeted to an aphid essential gene.

22. An aphid gene comprising the sequence of any one of SEQ ID NO:5 to 8, or SEQ ID NO:12.

23. The method of claim 18 or 19, wherein a cationic oligopeptide is mixed in the diet together with the dsRNA.

24. The method of claim 23, wherein said oligopeptide is a 12 amino acids poly-Arginine peptide.

25. The plant cell, tissue, plant or plant seed of claims 9 or 17, which also comprises a chimeric gene encoding a cationic oligopeptide.

26. The plant cell, tissue, plant or plant seed of claim 25, wherein said oligopeptide is a 12 amino acids poly-Arginine peptide.

<110> Commonwealth Scientific and Industrial Research Organisation

Bayer BioScience NV

Waterhouse, Peter

Whyard, Steven

Van Rie, Jeroen

<120> Insect resistance using inhibition of gene expression

<130> BCS03-2008 WO1

<150> US 60/520,306

<151> 2003-11-17

<160> 12

<170> PatentIn version 3.0

<210> 1

<211> 27

<212> DNA

<213> artificial

<220>

<223> designed degenerate primer

<400> 1

aaaacagaag aagaggtaaa aaygara

27

<210> 2

<211> 28

<212> DNA

<213> artificial

bcs03-2008.ST25

<220>

<223> designed degenerate primer

<220>

<221> misc_feature

<223> n at 20 is c, g, a or t

<400> 2

ggtttctggc ttcgtctggn gtrtaytt

28

<210> 3

<211> 20

<212> DNA

<213> artificial

<220>

<223> designed degenerate primer

<400> 3

tacaactcsa tcytgaccac

20

<210> 4

<211> 22

<212> DNA

<213> artificial

<220>

<223> designed degenerate primer

<400> 4

tccatrccyt cwccbacrta cc

22

<210> 5

<211> 279

<212> DNA

<213> Aphis gossypii

bcs03-2008.ST25

<400> 5
 aaaacagaag aagaggtaaa aatgagaatg aaacagaaaa gcgtgagttg gtgttcaaag 60
 aagatggaca agaatatgct caagttacca aaatggtggg aaatggacgt ctagaagcaa 120
 tgtgttttga tgggtgaaga cgactttgtc acattcgagg aaaacttagg aaaaagggtg 180
 ggatcaatca agctgacata gtattgatag gcttacgtga atatcaagat acaaaagccg 240
 atgtaatttt gaaatacacc ccagacgaag ccagaaacc 279

<210> 6

<211> 279

<212> DNA

<213> *Myzus persicae*

<400> 6
 aaaacagaag aagaggtaaa aacgaaaatg aaaccgagaa gcgtgaattg gtgttcaaag 60
 aagatggcca agaatatgct caagttacca aaatggtggg aaatggacgt ctagaagcta 120
 tgtgctttga tgggtgttaa cgactttgcc acatacgagg aaaacttagg aaaaagggtat 180
 ggattaatca agctgatata gtattaatag gtttacgtga ataccaagac acaaaagccg 240
 atgtaatttt gaaatacaca ccagacgaag ccagaaacc 279

<210> 7

<211> 638

<212> DNA

<213> *Aphis gossypii*

<220>

<221> misc_feature

<223> n at 591, 592 and 637 is a, c, g or t

<400> 7
 tcatggctgg actacgaggc catctacgac atctgccgcc ggaacctgga catcgagcgg 60
 cccacgtaca cgaacctcaa ccggctgacg gggcagatcg tgctggtccc atcacgggcg 120
 tcgctgcggt tcgacggcgc gctgaacgtc gacctgaccg agttccagac gaacctgggtg 180
 ccgtacccgc gcattcactt cccgctggcc acgtacgcgc cggtcataatc ggccgagaag 240

bcs03-2008.ST25

```

gcgtaccacg agcagctgtc cgtggccgaa atcaacccaa cgcgtgcttc gaaccggcca 300
aaccagaatg ggtcaagatg cgaccgcgg cacggcaagt acatgggcct gctgcaatgc 360
tgtaaccgcg gcgacgtcgt gcccaaggac atgaacgcgg ccatcgccac catcaagacc 420
aagaggacca tcgtgtacgt cgactggtgc ccgaccgggt tcaagggtggg catctactac 480
cagccgccga ccgtggtgcc gggggcgatc tggccaaggt gcagcgggcg gtgtgcatgt 540
tgtccaacac gacggccatc tccgaggcgt gggcccggct cgaccacaag nntgacctga 600
tgtacgtga cagcgcgtc cgtccactgg tacgtang 638

```

<210> 8

<211> 628

<212> DNA

<213> *Myzus persicae*

<220>

<221> misc_feature

<223> n at 3, 113, 128, 137, 509, 615, 617, and 627 is a, c, g, or t

```

<400> 8
tgnacacagt gactgtgcat tcatggtcga taatgaagcc atctatgaca tctgccgtcg 60
taatctcgat attgaacgtg cccacttaca ctaacttgaa tcgtcttatt gncagattg 120
tgtcttcat cacagctct ctccgtttcg atgggtgccct caatgttgac ttgactgaat 180
tccagaccaa tttggtccca tcccccgta ttcatttccc attggtcact tatgcaccag 240
tcattctccg tgaaaaggct taccatgaac aattgtccgt atcagaaatc actaacgctt 300
gttttgaaac agccaaccaa atggtgaaat gtgatccacg tcatggcaaa tacatggctt 360
gttgcatgtt gtaccgtggt gatgtgtac ccaaagacgt caacgtgcc attgcttcca 420
tcaagaccaa gagaacattc agtttgttga ctggtgtcca actggtttca aagttgggta 480
tcaactacca accccaacc gtggtaccng gtgtgacttg gtctaaagta caacgtgctg 540
tctgcatgtt gtccaacact acagctattg ctgaagcttg ggtctagggt tggtaccaca 600
agttcgtaac ttgcnantac gtccacna 628

```

<210> 9

<211> 30

<212> DNA

bcs03-2008.ST25

<213> artificial

<220>

<223> designed primer sequence

<400> 9

cccaagcttt gcctggtgtg tggcgaccgg

30

<210> 10

<211> 30

<212> DNA

<213> artificial

<220>

<223> designed primer sequence

<400> 10

cccaagctta tcctggaaat agacaagtcg

30

<210> 11

<211> 408

<212> DNA

<213> Myzus persicae

<400> 11

tcgtccggtt accattacaa cgctctcaca tgcgaaggat gcaagggggtt cttccggagg	60
agcatcacca agaacgccgt gtaccagtgc aagtacggca acaattgcga aatcgacatg	120
tacatgaggc ggaagtgccg ggagtgccgg ctgaaaaaat gcctgaccgt cggcatgagg	180
cctgaatgtg ttgtacctga agttcaatgc gcagtaaaaa gaaaggagaa aaaagctcaa	240
cgagaaaaag ataaaccaa ttctactaca gacatttctc ctgaaataat aaaaatagaa	300
cctacagaga tgaagattga atgtggtgaa ccaatgataa tgggcacacc tatgccgact	360
gtaccttacg tgaaaccttt gagttctgaa caaaaagaac tgatccac	408

<210> 12

<211> 1173

<212> DNA

<213> Myzus persicae

bcs03-2008.ST25

<220>

<221> misc_feature

<223> n at 704 is c, g, a, or t

<400> 12
 tgccagcgca tttgcgacca gtgggacaga ttaggtagct tgacacagaa acggagaact 60
 gacttggatg atgcagaaaa aatattagag aaaattgata tattgcattt ggaattcgct 120
 aagagagcag ctcttttcaa caactggttg gatggtacac gtgaagattt agtggacatg 180
 ttcatgttac aactgtttga ggaaatccaa ggattgattg atgcacatgg acaatttaag 240
 gctactttgt ctgatgctga caaagagtac aactctatca ttggactggt caaagatggt 300
 gagtcaactg tacaaaaata ccaaatacct ggtggtcttc agaaccgta cactactttg 360
 acttctagtg atttaagcaa aaaatggtct gaagtgaac atttagtgcc ccaaagagac 420
 acgaccctcc aagctgaact cagaaaacaa caaacaatg agatgctacg tcgtcaattt 480
 gcggagaagt caaatcaagt gggctccttg attgagaggc aaatggacgc tgtcacggcc 540
 atcggtatgg gattgcaggt tctctggaag atcaattgca ccaactgaaa caatttaggg 600
 ctactttgtc tgatgctgac aagagtacaa ctctatcatt ggactggtca agatgttgag 660
 tcaactgtac aaaaatacca aatacctggt ggtcttcaga accngtacac tactttgact 720
 tctagtgatt taagcaaaaa atggtctgaa gtgaaacatt tagtgcccca aagagacacg 780
 accctccaag ctgaactcag aaaacaacaa aacaatgaga tgctacgtcg tcaatttgcg 840
 gagaagtcaa atcaagtggg tccttggatt gagaggcaaa tggacgctgt cacggccatc 900
 ggtatgggat tgcaagggtc tctggaagat caattgcacc aactgaaaca atacgaacag 960
 aatgtgtttg catacaagcc acatattgag gaattagaga aaatccacca agctgtacaa 1020
 gagggatga tcttcgaaaa caggtatact caatacacia tggagacatt acgtgttggg 1080
 tgggaacaac tattgacgtc cataaatcgc aatgtgaatg aagtagaaaa ccaaattattg 1140
 accagagact ccaaaggcat cacccaggag cag 1173